The Structure of Melanoregulin Reveals a Role for Cholesterol Recognition in the Protein's Ability to Promote Dynein Function

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Supplementary Materials

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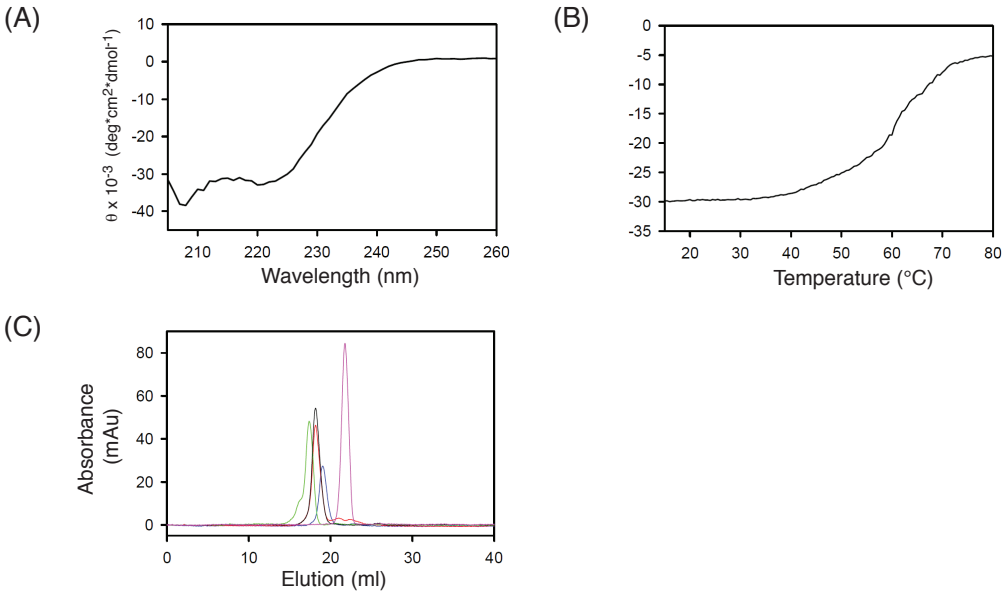

Figure S1. Related to Figures 1 and 2 **(A)** A Circular Dichroism spectrum reveals the predominantly α-helical conformation of MregΔ32. **(B)** The temperature dependence of the CD spectra monitored at 220 nm indicates that after 35 °C, MregΔ32 starts changing its conformation, with a major transition at about 60 °C. Therefore, all the NMR experiments were carried out at 29 °C, far from MregΔ32's initial transition temperature. **(C)** Size exclusion chromatography of MregΔ32 shows its monomeric state, although it runs at a slightly higher molecular weight, suggesting the presence of some unstructured regions. The FPLC peaks yield a MW of 21.33 kDa (black) for MregΔ32 based on the marker proteins used to calibrate the sizing column (43 kDa (green), 29 kDa (red), 13.7 kDa (blue), and acetone peak (magenta)).

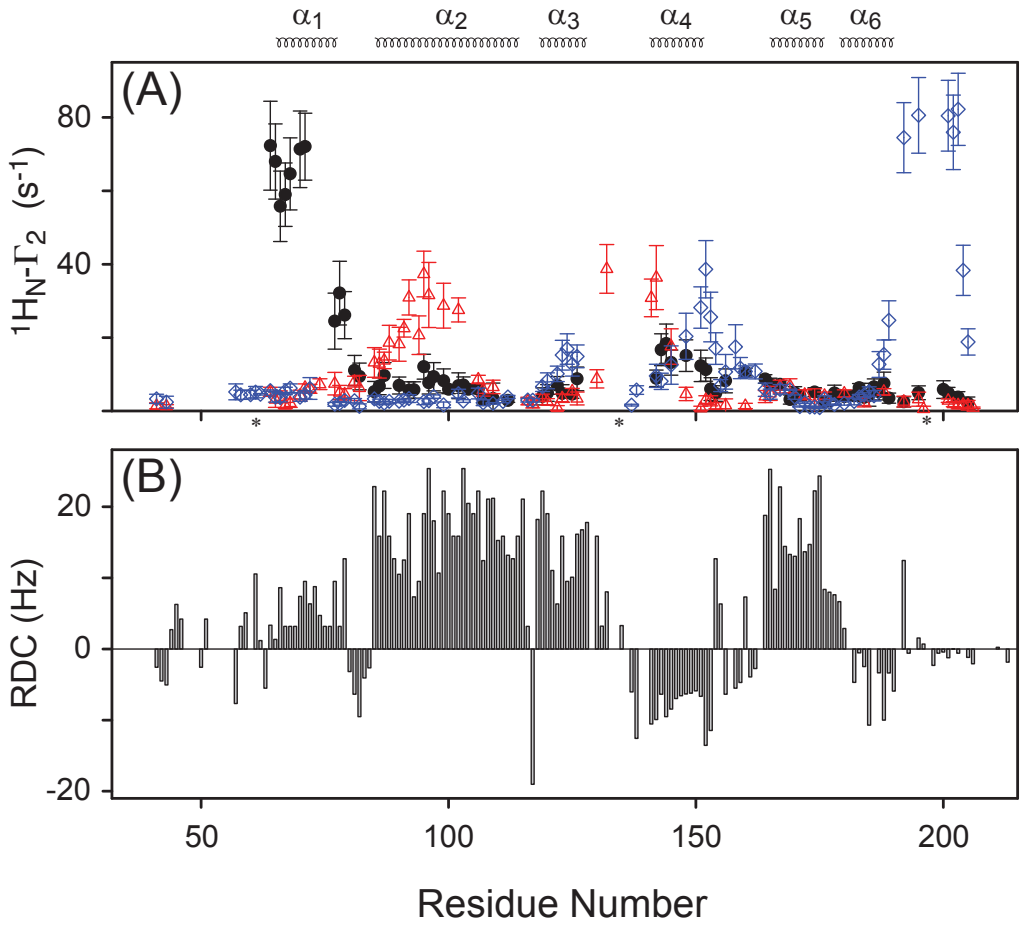

Figure S2. Related to Figure 3, Paramagnetic Relaxation Enhancement (PRE) and Residual Dipolar Coupling (RDC) data for MregΔ32 that were used in the NMR structure calculations. **(A)** PRE values shown as a function of residue number in MregΔ32 with spin probe proxyl attached to C60 (black circle), C136 (red triangle) and C198 (blue

diamond). **(B)** RDC of ^{15}N-labeled MregΔ32 measured in the presence of 10mg/ml of Pf1 phage at 29 °C.

Figure S3. Related to Figure 4, alignments of multiple Mreg sequences made using the CLUSTAL W 1.83 web server. Shown are the primary sequences of Mreg from Mouse, Rat, Bovine, Human, Latimeria, and Xenopus. The predicted CRAC motif in these sequences is highlighted in green, while the highly conserved tyrosine in this motif, which is important for cholesterol binding, is colored in red. The symbol "*" denotes a position where all the sequences have the same amino acid, the symbol ":" denotes a position where all the sequences have an amino acid of the same chemical type, and the symbol "." denotes a position where all the sequences show some similarity in sequence alignment.

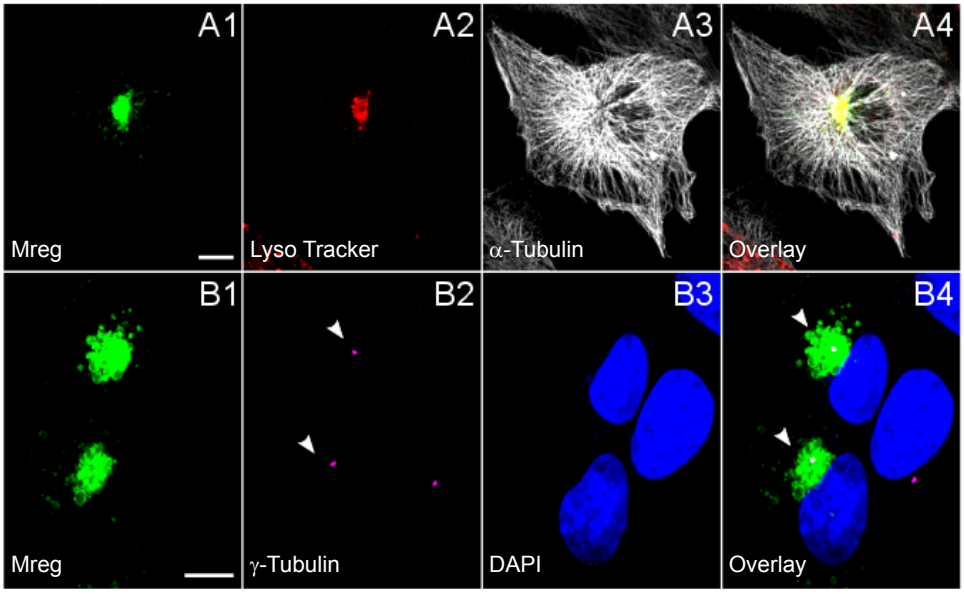

Figure S4. Related to Figure 5, the over expression of Mreg drives the accumulation of late endosomes/lysosomes at the MTOC. Panels **A1-A4** show that Neon-tagged Mreg (Panel A1) targets to LysoTracker Red-positive late endosomes/lysosomes (Panels A2) that accumulate at the minus ends of the interphase microtubule array (i.e. the MTOC), as revealed by staining for α-tubulin (Panel A3 and the Overlay in Panel A4). Panels **B1-B4** show that late endosomes/lysosomes labeled with Neon-tagged Mreg (Panel B1) accumulate around the γ-tubulin-positive MTOC (red dots in Panel B2; see arrowheads) that lie immediately adjacent to the DAPI-stained nucleus in the cell center (Panel B3 and the Overlay in Panel B4). These images are representative of ~30 cells examined. Mag bar = 10 µm.

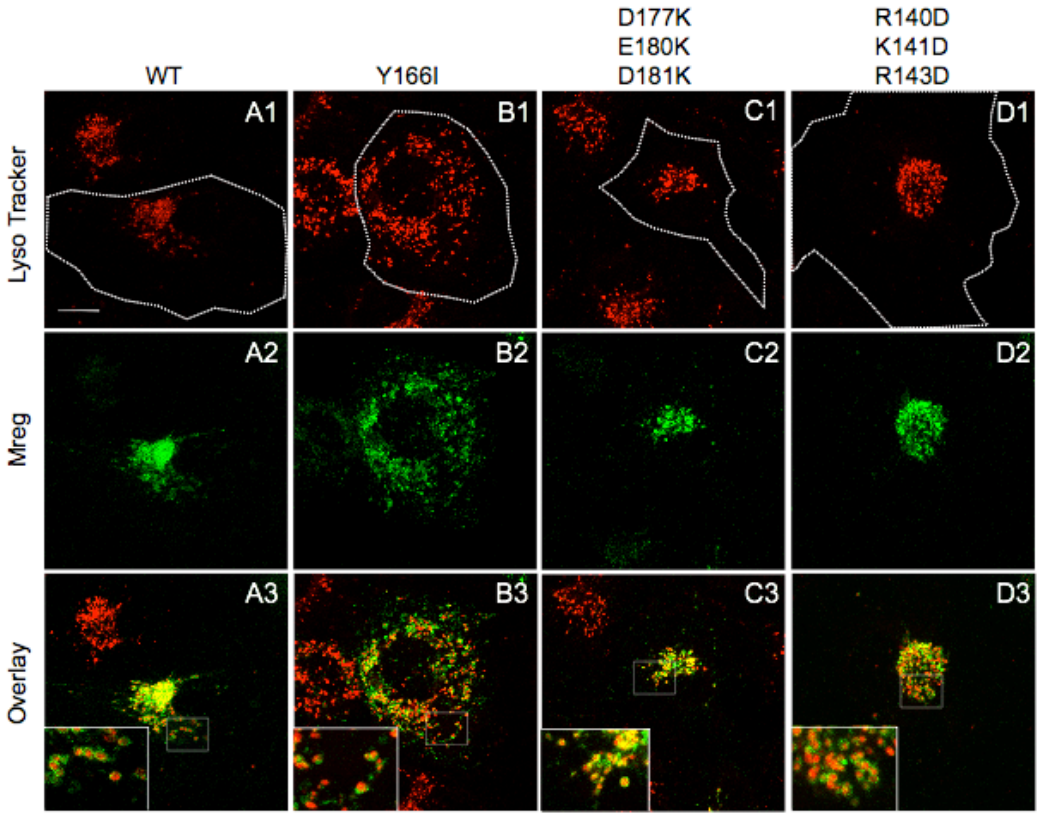

Figure S5. Related to Figure 5, Mreg targets to LysoTracker-positive late endosomes and lysosomes. Shown are high magnification images of cells 18 hours after transfection with Neon-tagged WT Mreg (**A1-A3**), Neon-tagged Mreg containing the Y166I mutation (**B1-B3**), Neon-tagged Mreg containing the D177K, E180K, D181K mutations (**C1-C3**), or Neon-tagged Mreg containing the R140D, K141D, R143D mutations (**D1-D3**). The signal for LysoTracker Red is shown in Panels A1, B1, C1, and D1 (the thin dotted line shows the cell outline). The signal for Neon-tagged Mreg is shown in Panels A2, B2, C2, and D2. The overlaid image, as well as a magnified inset corresponding to the region boxed in white, is shown in Panels A3, B3, C3, and D3. Note that, as expected from the data in Figure 5, Neon-tagged Mreg containing the Y166I mutation does not cause the accumulation of late endosome/lysosomes in the cell center (B1-B3). These images are representative of ~20 cells examined. Mag bar = 10 µm.

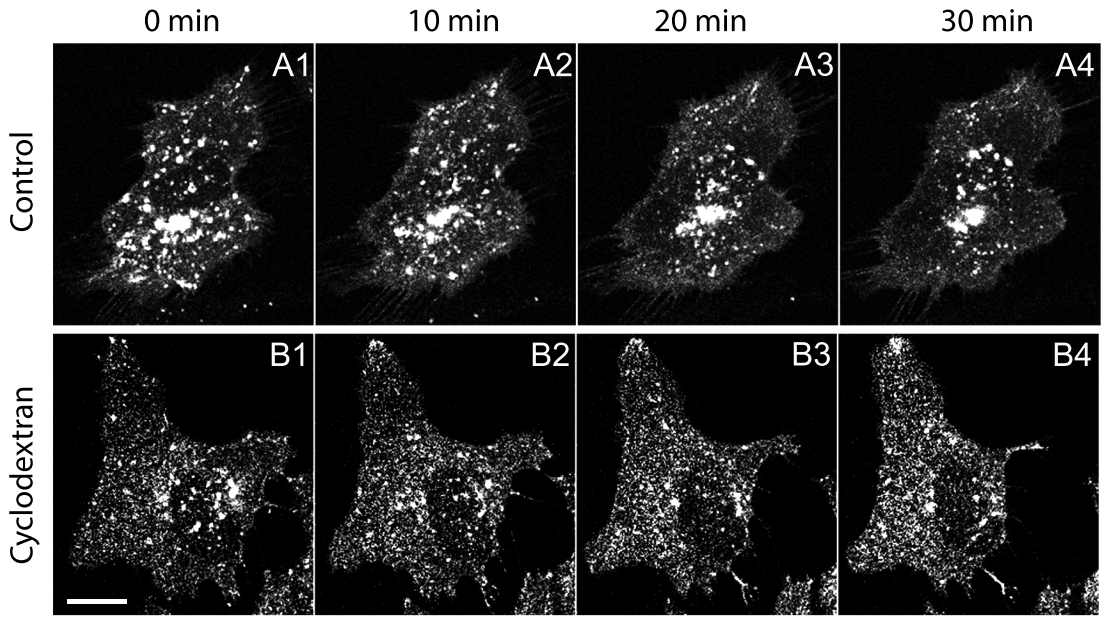

Figure S6. Related to Figures 4 and 5, Transient depletion of cholesterol from cellular membranes using methyl-β-cyclodextran (CD) blocks the ability of Mreg over-expression to promote the accumulation of late endosomes/lysosomes at the MTOC. Panels A1-A4 show a representative CV1 cell that had been transfected with WT Mreg 18 hours prior to accumulate late/endosomes at its MTOC, treated for 120 minutes with 5 µg/ml nocodazole to disassemble its microtubule cytoskeleton (causing its late endosomes/lysosomes to re-spread) and the carrier used for CD, washed with complete media containing carrier and LysoTracker, and imaged for 30 min at 30 sec/frame (see also Supplementary Movie left panel). Panels B1-B4 show a representative CV1 cell that had been transfected with WT Mreg 18 hours prior, treated for 120 minutes with 5 µg/ml nocodazole and 10 mM CD to deplete cholesterol from its membranes, washed with complete media containing 10 mM CD and LysoTracker, and imaged for 30 min at 30 sec/frame (see also Supplementary Movie right panel). Shown are the distributions of late endosomes/lysosomes at 0, 10, 20, and 30 min for both cells. These results are representative of three cells imaged for each condition. Mag bars: 10 µm.